activation of the voltage sensors in the fourth transmembrane segment (S4) is transduced into pore opening via coupling of the S4-S5 linker to the C-terminal S6 segment. In TRPA1, the gain-of-function mutation N855S located in the S4-S5 region has been associated with familial episodic pain syndrome. In an attempt to elucidate the role of the S4-S5 linker and its putative interaction(s) with S6 or the first C-terminal helix in the voltage-dependent gating of TRPA1, we used site-directed mutagenesis, whole-cell electrophysiology, single-channel recording, and molecular dynamics simulations. The charge-reversal mutations K868E and K969E resulted in a decrease in the rectification index compared to wild-type TRPA1 channels, and a virtually voltage-independent conductance-voltage (G-V) relationship. This effect was also observed in the adjacent charge-neutralizing mutant H970A, but was less pronounced in charge- reversal H970D. These results indicate that positively charged residues in the S4-S5 linker and the helix adjacent to the C-terminal S6 segment play a vital role in the voltage-dependent gating of TRPA1.

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**2322-Pos Board B341**

**Ultraviolet Light Phototransduction Activates TRPA1 to Mediate Melanin Synthesis in Human Melanocytes**

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Human skin is constantly exposed to solar ultraviolet radiation (UVR), the most prevalent environmental carcinogen. Humans have the unique ability among mammals to respond to UVR by increasing their skin pigmentation, a protective process driven by melanin synthesis in epidermal melanocytes. However, the mechanism used by melanocytes to detect and respond to UVR is not well understood. Here we report that transient receptor potential A1 (TRPA1) ion channels expressed in human melanocytes are activated by UVR and mediate early melanin synthesis. We show that in human epidermal melanocytes (HEMs) physiological doses of UVR activate a retinal-dependent current mediated by TRPA1. The UVR photocurrent density was reduced by TRPA1 antagonists and abolished in HEMs expressing TRPA1-targeted miRNA. The TRPA1 photocurrent is UVA specific and requires G protein signaling, providing the first evidence for TRPA1 function in mammalian phototransduction. In HEMs, TRPA1 activation contributes to UVR-induced calcium responses to mediate downstream cellular effects. Remarkably, the UVR-induced and retinal-dependent early increase in cellular melanin content was significantly reduced in HEMs treated with TRPA1 antagonists and abolished in HEMs expressing TRPA1-targeted miRNA, suggesting that TRPA1 is required for early melanin synthesis. Our results show that TRPA1 is essential for a novel extra-ocular phototransduction pathway in human melanocytes that is activated by physiological doses of UVR and results in early melanin synthesis.

**2323-Pos Board B342**

**Characterization of Small Molecule TRPC3 and TRPC6 agonist and Antagonists**

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Canonical transient receptor potential channels (TRPC3/6) are mechano-sensitive, receptor- and store-operated channels that mediate $Ca^{2+}/Na^{+}$ influx into cells. TRPC3/6 are implicated in the regulation of vascular tone, cell growth, proliferation and pathological hypertrophy. Potent and selective small molecule TRPC3/6 agonist and antagonist tools had been lacking to study the functions of TRPC3/6. We report here potent, small molecule agonist (GSK1702934A) and antagonists (GSK417651A and GSK2293017A) of TRPC3/6. Whole-cell patch-clamp experiments demonstrated that GSK1702934A activated TRPC3 and TRPC6 current in HEK293 cells transduced with recombinant human TRPC3 or TRPC6 with an $EC_{50}$ of ~ 0.08 μM and 0.44 μM, respectively. GSK417651A inhibited both TRPC3 and TRPC6 current with $IC_{50}$s of ~ 0.04 μM. A more potent TRPC3/6 antagonist GSK2293017A exhibited an $IC_{50}$ of ~ 0.01 μM. GSK417651A was likely acting on TRPC3/6 from the extracellular side, because dialyzing the cells with 10 μM GSK417651A in the pipette solution did not prevent the activation of TRPC6. In the rat cardiomyocyte like cell line H9C2, GSK1702934A (1 μM) activated TRPC6-like current which was completely blocked by GSK417651A (1 μM). TRPC3/6 activator GSK1702934A (1 μM) transiently increased the perfusion pressure of isolated rat heart retrogradely perfused via aortic cannulation. This effect was completely eliminated by pretreatment with the TRPC3/6 blocker GSK2293017A (1 μM). IV bolus of GSK1702934A (0.3 ~ 3 mg/kg) transiently increased blood pressure by 15 ~ 35 mmHg in conscious Sprague Dawley rats. This blood pressure effect was dose-dependently inhibited by pretreatment of GSK2293017A (2.5 ~ 250 μg/kg/min infusion for 30 min) with complete inhibition occurring at a free plasma concentration of 0.81 μM. Therefore, these small molecule agonist and antagonists could be useful tools to help understand the characteristics and functions of TRPC3/6 in tissues and animals.

**2324-Pos Board B343**

**$PIP_2$ Dynamics underlying Muscarinic or Vasopressin Receptor-Activated TRPC3 C6 And C7 Currents**

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Subfamily of human expressed TRPC channels (TRPC3/6/7) are activated by 'diacylglycerol' (DAG), a phospholipase C (PLC) hydrolyzed product of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] (Hofmann *et al*., 1999, Nature). In contrary, we have recently reported that the depletion of $PI(4,5)P_2$ by-itself act as a potent negative regulator to all these channels even in the presence of DAG (Imai *et al*., 2012, J. of Physiol.). Stimulation upon the vasoconstrictile receptors coupled with PLC theoretically causes inseparable bimodal effect to these TRPC channels, i.e. activation and inhibition by DAG production and coincident $PI(4,5)P_2$ reduction or depletion which is corresponding to the strength of PLC activities. Here, to elucidate such self-limiting regulatory function coupled to $PI(4,5)P_2$-DAG signal, we simultaneously measure TRPC6/7 currents in the whole-cell configuration and $PI(4,5)P_2$ dynamics by FRET using $PI(4,5)P_2$ binding PH-domain sensor proteins in the various strength of muscarinic- or vasopressin-receptor stimulation. Our simultaneous detection approach reveals good kinetics correlation between TRPC activation/fast-inactivation and $PI(4,5)P_2$ depletion. Furthermore, a simple self-limiting regulation model wherein experimentally determined $PI(4,5)P_2$ binding constants incorporated aware an emergence of fast recovery of $PI(4,5)P_2$ to produce slow inactivation (plateau phase) of TRPC currents. We find that such model implicated $PI(4,5)P_2$ enhancement can be reproduced after the fast $PI(4,5)P_2$ depletion under the robust agonist stimulation by the local FRET measurement near the channels. Hence, these data indicates that self-limiting regulation coupled to $PI(4,5)P_2$-DAG signal is the pivotal mechanism to understand the receptor-PLC mediated TRPC3/6/7 channels activity.

**2325-Pos Board B344**

**RASD1 Independently Activates TRPC4 through Gαi of GPCR**

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Canonical transient receptor potential (TRPC) channels have six transmembrane (6-TM) domains and are $Ca^{2+}$-permeable and non-selective cation channels. It is generally speculated that TRPC channels are activated by stimulation of Gq-PLC-coupled receptors and oxidation. Activator of G-protein signaling1 (AGS1 or RASD1), the ras-related protein, interacts with Gi/Go and activates heterotrimeric G-protein signaling systems independent of G-protein-coupled receptor (GPCR). It is previously reported that AGS1 is related to GIRK channel and $Ca^{2+}$ channel. However it is unknown whether AGS1 is associated with TRPC channels. We assumed that AGS1 might regulate TRPC4 channel, since AGS1 interacts with Gi/Go and TRPC4 is activated by Gi/o subunits. Here, we measured whole cell current of TRPC4/5 after the co-expression of TRPC4 or TRPC5 with constitutively active form of small GTPases in HEK293 cells. AGS1 (CA) mutant (Q to L) activated TRPC4 (38.8 ± 7.2 pA/pF) without GTPγS and independently of GPCR. Pertussis toxin (PTX), $G\alpha_i$ specific inhibitor, blocked RASD1-activated TRPC4 current (3.4 ± 1.6 pA/pF). When co-expressed with dominant negative $G\alpha_i$ protein subtype, TRPC4 activation by RASD1 was completely inhibited. With previous report that TRPC4 are activated primarily by selective Gαi subunits rather than $G\alpha_q$, these results suggest that AGS1 activates TRPC4 channel through modulating Gαi subunits and AGS1 is a new activator for TRPC4 channel.

**2326-Pos Board B345**

**An Essential Role of $PI(4,5)P_2$ for Maintaining the Activity of the Transient Receptor Potential Canonical (TRPC)4 Channel TRPC4β**

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The Transient Receptor Potential Canonical 4 (TRPC4) channel is a $Ca^{2+}$-permeable, non-selective cation channel in mammalian cells and mediates